

Host plant suitability of *Solanum japonense* (Solanaceae) as an alternative larval food for three closely related *Epilachna* ladybird beetles (Coleoptera: Coccinellidae)

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Abstract

The host plant suitability of the solanaceous wild herb *Solanum japonense* as an alternative larval food for the three non-pest species of the *Epilachna vigintioctomaculata* complex, namely *E. niponica*, *E. pustulosa*, and *E. yasutomii*, was investigated under laboratory conditions. Three larval developmental traits (eclosion rate, developmental duration, and body size) were recorded together with the leaf area consumed throughout the developmental stages. All three ladybird species showed sufficient performance on *S. japonense*, although the suitability of *S. japonense* for larval development appeared to be highest for *E. pustulosa* and lowest for *E. yasutomii*. The measurements of leaf consumption revealed that *E. niponica* and *E. pustulosa* consumed a significantly larger leaf area of *S. japonense* than did *E. yasutomii* during the developmental stages. However, when the influence of interspecific body size difference was removed, leaf consumption was significantly smaller in *E. niponica* than it was in the other two species, suggesting a higher feeding efficiency of *E. niponica* upon digesting *S. japonense* leaves.

Key words: *Epilachna niponica*, *Epilachna pustulosa*, *Epilachna yasutomii*, larval performance, *Solanum japonense*

INTRODUCTION

The *Epilachna vigintioctomaculata* complex, a group of herbivorous ladybird beetles composed of four closely related species, differs greatly in external morphology and food plants in and around the Japanese Archipelago. Among the members of this complex, three species other than *E. vigintioctomaculata* Motschulsky, namely *E. niponica* Lewis, *E. pustulosa* Kôno and *E. yasutomii* (Katakura), are particularly closely related to each other (Katakura, 1981; Tsurusaki et al., 1993; Kobayashi et al., 1998). These three species are regarded as non-pest species; they usually occur on wild native plants, thistles (*Cirsium* spp.: Asteraceae) and/or blue cohosh (*Caulophyllum robustum* Maxim.: Berberidaceae), whereas *E. vigintioctomaculata* is a notorious pest of solanaceous crops (reviewed in Katakura, 1997).

The subfamily Epilachninae as a whole has a strong relationship to solanaceous and cucurbitaceous plants (Schaefer, 1983), and it was strongly suggested by mtDNA analysis that solanaceous

plants are an ancestral host of the *E. vigintioctomaculata* complex (Kobayashi et al., 1998). Of relevance, although the three species of the *E. vigintioctomaculata* complex other than *E. vigintioctomaculata* are basically non-pest species, their invasion to solanaceous crop fields (mainly the potato *Solanum tuberosum* L.) has been reported repeatedly (Katakura, 1981; Hoshikawa, 1983; Shirai, 1987; Yamauchi, 1994). Furthermore, in a few populations, some or most larvae completed their development on potato leaves in crop fields (Katakura, 1981; Shirai and Morimoto, 1997, 1999). Therefore, these three closely related ladybird species serve as good arenas for investigation of the process of pest establishment, as well as for studies concerning more general issues in phytophagous insects, including diet breadth evolution and host shift (cf. Katakura, 1997). For example, Shirai and Morimoto (1997, 1999) compared life history traits between pest and non-pest populations of *E. niponica* and *E. yasutomii*.

In both applied and basic studies, especially for laboratory experiments, artificial diets are often

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particularly convenient for preparing sufficient numbers of insects that are reared under an identical food condition, or for rearing of insects under different environmental conditions other than the food condition. However, artificial diets that support the complete larval growth for ladybird beetles belonging to the subfamily Epilachninae, including the *E. vigintioctomaculata* complex, have not yet been developed (cf. Kogan, 1971; Murata et al., 1994). Hence, the leaves of some solanaceous plants have thus far been used as alternative foods for larvae of the members of the *E. vigintioctomaculata* complex, e.g., potato (Katakura et al., 1989), *Solanum japonense* Nakai (Katakura and Hosogai, 1997), and *S. megacarpum* Koidz. (Katakura et al., 1981). Even though sufficient larval performance was obtained on these alternative plants in the studies cited above, the suitability of these plants has been identified mainly by experience. In particular, the suitability of *S. japonense* and *S. megacarpum* has not yet been precisely studied under laboratory conditions.

In the present study, we assessed the suitability of a perennial wild herb, *S. japonense*, as an alternative larval food for the three non-pest species of the *E. vigintioctomaculata* complex. On the basis of the results of larval rearing in the laboratory, we considered whether *S. japonense* is similarly suitable for the three ladybird species through interspecific comparison on larval developmental performance and feeding efficiency.

MATERIALS AND METHODS

Insect materials. In the late spring of 1997, we collected posthibernating adult beetles of the three epilachnine species on their respective host plants in Hokkaido, northern Japan. The sampling localities and plants from which beetles were collected were as follows: *E. niponica*, near Junsai-numa in Oshima District, on *Cirsium alpicola* Nakai; *E. pustulosa*, at Sankakuyama in Sapporo, on *C. kamtschaticum* Ledeb.; and *E. yasutomii*, near Konuma in Oshima District, on blue cohosh.

Rearing of larvae. For each epilachnine species, three to five females and males were confined as pairs in separate styrene cases (5.5 cm×6.0 cm×2.0 cm) for mating. Virtually all females would have copulated and stored sperm before overwintering (cf. Katakura, 1982), and additional copula-

tions could also have occurred in the late spring before collection. Hence, we started to gather egg masses after we had confirmed at least three copulations for each pair in order to minimize genetic variation among offspring from each pair. Last male sperm precedence (P2) in *E. pustulosa* was reported as 0.651–0.827 (Nakano, 1985). Assuming the same sperm precedence pattern for the last three successive copulations, the expected fertilization success of previous males is $0.349^3 = 0.0425$ at most. For all three species, we selected one pair from each, the female of which showed normal fertility; these three pairs then served as the parents of larvae subjected to rearing.

For each species, a total of 15 larvae (five larvae derived from each of three different egg masses) were reared individually in styrene cases (8.0 cm×15.5 cm×3.0 cm), the bottom of which was covered with moist filter paper. Rearing was carried out under a controlled regime of 16L–8D at 20°C. Throughout the experimental period, we provided a sufficient amount of *S. japonense* leaves picked every day or every other day. Leaves of potted plants raised from cuttings in a greenhouse were used. All of the cuttings used belong to the same clone, derived from an individual of *S. japonense* that originally grew on the campus of Hokkaido University and was transplanted into an experimental garden on the same campus approximately a dozen years ago.

The leaf area consumed by each larva was measured throughout the developmental stages (from hatching to pupation) with the aid of image processing software (NIH Image ver. 1.55; National Institute of Health, Bethesda, MD, USA), after being photocopied and scanned into a computer. Larvae were reared until emergence. We recorded the eclosion rate (i.e., the percentage of individuals reaching adulthood), the developmental duration (the number of days from hatching to adult eclosion), and the pronotum width of newly emerged adults as the body size index.

Comparison of leaf consumption. In order to examine the suitability of *S. japonense* for the three epilachnine species with respect to feeding efficiency, leaf consumption during the developmental stages was compared among beetle species. Close relationships between leaf consumption and developmental traits were expected; it remains uncertain which factor, leaf consumption or develop-

Table 1. Developmental traits [mean±SE (*n*)] of three *Epilachna* species reared on *Solanum japonense*

Species	Eclosion rate in %	Developmental duration in days	Pronotum width in mm	
			Female	Male
<i>Epilachna niponica</i>	100.0 (15/15)	31.00±0.195 (15) a	4.01±0.026 (8) a	3.81±0.024 (7) a
<i>Epilachna pustulosa</i>	93.3 (14/15)	29.29±0.163 (14) b	3.67±0.029 (5) b	3.51±0.024 (9) b
<i>Epilachna yasutomii</i>	100.0 (15/15)	28.60±0.131 (15) c	3.28±0.015 (4) c	3.15±0.018 (11) c

Figures with different letters in the same column are significantly different ($p < 0.05$, Scheffé's method after log-transformation).

Table 2. ANOVAs for developmental traits of three *Epilachna* species reared on *Solanum japonense*

Source	df	Developmental duration		Pronotum width	
		SS	<i>F</i>	SS	<i>F</i>
Species	2	0.0466	56.159***	0.2498	384.634***
Sex	1	0.0003	0.751	0.0195	59.958***
Species×Sex	2	0.0022	2.634	0.0002	0.357
Error	38	0.0158		0.0123	

Data were log-transformed before the analyses. *** $p < 0.001$.

mental trait, is determinant of the other. Nevertheless, for the interspecific comparison, we assumed that leaf consumption during the developmental stages is largely determined by intrinsic developmental traits, because when a particular beetle species has a potentially longer developmental duration and/or larger body size, a larger leaf consumption can be expected. With this assumption, an interspecific comparison of feeding efficiency was conducted using an analysis of covariance (ANCOVA); developmental traits were considered as covariates.

RESULTS

Larval performance on *S. japonense*

During the rearing process, all of the larvae reached adulthood normally, except for one individual of *E. pustulosa* that died in the 4th (final) stadium period (Table 1). There were significant interspecific differences in developmental duration and body size (Tables 1, 2). Developmental duration was longest in *E. niponica* and shortest in *E. yasutomii*. *E. pustulosa* showed an intermediate value (Table 1). Body size was largest in *E. niponica*, smallest in *E. yasutomii*, and intermediate in *E. pustulosa* (Table 1).

Table 3. Total leaf area consumed (cm^2) during the developmental stages [mean±SE (*n*)] of three *Epilachna* species reared on *Solanum japonense*

Species	Female	Male
<i>Epilachna niponica</i>	50.4±1.08 (8) a	43.6±1.23 (7) a
<i>Epilachna pustulosa</i>	50.8±2.91 (5) a	47.0±1.86 (9) a
<i>Epilachna yasutomii</i>	38.5±0.78 (4) b	36.3±1.37 (11) b

Figures with different letters in the same column are significantly different ($p < 0.01$, Scheffé's method after log-transformation).

Leaf consumption and feeding efficiency

The total leaf area consumed during the developmental stages is given in Table 3. *E. niponica* and *E. pustulosa* consumed significantly larger amounts of *S. japonense* leaves than did *E. yasutomii* (Table 3).

Since an ANCOVA requires the slopes of the regression lines being fitted in each group to be parallel, i.e., the homogeneity of slopes of the lines (Sokal and Rohlf, 1995), we first regressed the leaf area consumed during the developmental stages onto two developmental traits, namely, developmental duration and pronotum width. For each beetle species, the leaf area was significantly regressed

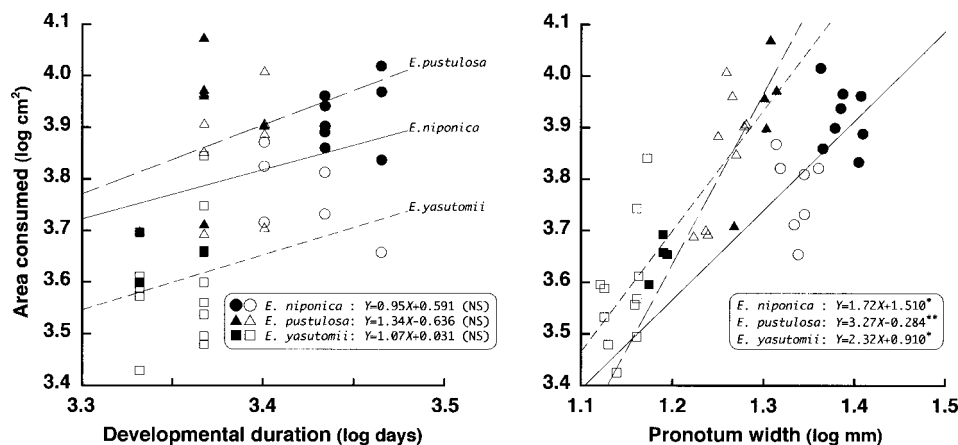


Fig. 1. Regressions of leaf area consumed during developmental stages (Y) onto two developmental traits (X) for three *Epilachna* species reared on *Solanum japonense*. Closed and open symbols indicate females and males, respectively. All data were log-transformed before the analyses. * $0.01 < p < 0.05$, ** $0.001 < p < 0.01$.

onto body size (Fig. 1), whereas regression of the leaf area onto developmental duration was not significant ($p > 0.05$ for all three regressions; Fig. 1). Hence, we employed pronotum width as the covariate to conduct an ANCOVA for further interspecific comparison of leaf consumption. The homogeneity of the slopes of the regression lines was examined by ANOVA for dependent variable with the covariate and the independent variable as sources of variance; if the independent variable \times covariate interaction term was not significant, the homogeneity of slopes was assured (SYSTAT Inc., 1992). In the ANOVA for leaf area consumed (dependent variable) by beetle species (independent variable) and pronotum width (covariate), the interaction term was not significant (after log-transformation, beetle species: $df=2$, $F=0.698$, $p > 0.05$, pronotum width: $df=1$, $F=23.086$, $p < 0.001$, beetle species \times pronotum width: $df=2$, $F=0.882$, $p > 0.05$), assuring the homogeneity of the slopes of the three regression lines.

The ANCOVA revealed that there existed significant interspecific difference of leaf consumption during the developmental stages, even after the influence of body size difference was accounted for (after log-transformation, $df=2$, $F=12.604$, $p < 0.001$). After that influence was removed, leaf consumption by *E. niponica* was significantly less than that of both *E. pustulosa* and *E. yasutomii* (Table 4).

Table 4. Logarithmically transformed area consumed during the developmental stages [mean \pm SE (n)] of three *Epilachna* species reared on *Solanum japonense*, adjusted by ANCOVA (pronotum width of emerged adults was set as the covariate)

Species	Area consumed (log-transformed)
<i>Epilachna niponica</i>	3.61 \pm 0.055 (15) a
<i>Epilachna pustulosa</i>	3.85 \pm 0.024 (14) b
<i>Epilachna yasutomii</i>	3.85 \pm 0.058 (15) b

Figures with different letters are significantly different [$p < 0.01$, T'-method of multiple comparison (Sokal and Rohlf, 1995)].

DISCUSSION

Suitability of *S. japonense* as larval food

In order to examine whether the larvae realized sufficient performance on *S. japonense*, we compared larval performance in the present study and that obtained in previous studies with larvae reared on their respective host plants (Table 5). All three beetle species showed a somewhat higher eclosion rate and shorter developmental duration on *S. japonense* than on the respective host plants (Tables 1, 5). The achieved body size on *S. japonense* was similar to that on *C. alpicola* for *E. niponica*. *E. pustulosa* and *E. yasutomii* showed a somewhat larger and smaller body size on *S. japonense*, respectively, when compared with the size achieved on their respective host plants (Tables 1, 5). When developmental duration and body size were considered simultaneously by comparison to those traits on the respective host plants, the suitability of *S.*

Table 5. Larval performance (mean) of three *Epilachna* species reared on respective host plants reported thus far

Beetle species	Reared on	Eclosion rate (%)	Developmental duration (days)	Pronotum width (mm)		Source ^b
				Female	Male	
<i>Epilachna niponica</i>	<i>Cirsium alpicola</i>	95.0	32.44	4.00	3.83	I
<i>Epilachna pustulosa</i>	<i>Cirsium kamtschaticum</i>	84.5	31.34	3.51	3.34	I
	<i>Caulophyllum robustum</i>	92.0	34.85	3.50	3.36	II
		98.0	33.94	3.45	3.33	II
<i>Epilachna yasutomii</i>	<i>Caulophyllum robustum</i>	88.0	35.34	3.57	3.43	II
		58.2	29.7/29.8 ^a	3.32	3.20	III

Only the rearings carried out under a controlled regime of 16L–8D at 20°C were referred.

^a Values for female/male.

^b I, Koizumi et al. (1999); II, Fujiyama and Katakura (1997); III, Katakura and Hosogai (1994).

japonense appeared to be highest in the case of *E. pustulosa*, eliciting a relatively shorter developmental duration and larger body size. *S. japonense* for *E. yasutomii* would be ranked as least suitable due to the resulting decrease in expected body size.

Although small body size on *S. japonense* was expected for *E. yasutomii*, this result does not necessarily indicate inferior performance. If larval performance had deteriorated, a decrease in body size would be accompanied by prolonged developmental duration (cf. Fujiyama and Katakura, 1997), which was not the case in the present study (Tables 1, 5).

The measurement of leaf consumption revealed that *E. niponica* and *E. pustulosa* consumed a significantly larger leaf area of *S. japonense* during their developmental stages than did *E. yasutomii* (Table 3). However, when the influence of interspecific differences in body size (Table 1) was removed by ANCOVA, leaf consumption was significantly smaller in *E. niponica* than in the other two species (Table 4). This finding indicates that *E. niponica* required smaller amounts of *S. japonense* leaves than did the other two species in order to achieve a particular body size, strongly suggesting higher feeding efficiency of *E. niponica* upon digesting *S. japonense* leaves. The causal factor of this interspecific difference in feeding efficiency is unclear at present. It may be related to different adaptations to the current respective host plants, or it may have been shaped through historical changes in host use patterns that may differ among ladybird species and/or populations.

In general, it is concluded that *S. japonense* is sufficient as an alternative larval food for the three

non-pest species of the *E. vigintioctomaculata* complex.

Application of *S. japonense* as an alternative larval food

One major advantage of using *S. japonense* as an alternative food would be that this plant can be easily cultivated by multiplication from cuttings. Neither the respective host plants of the three epilachnine species (thistles and/or blue cohosh), nor the potato plant which also usually allows sufficient larval performance for all of members of the *E. vigintioctomaculata* complex (Katakura, 1981), are as easily cultivated as *S. japonense*. Moreover, *S. japonense* can be regarded as an appropriate alternative food from two additional points of view: (1) the family Solanaceae, to which *S. japonense* belongs, is taxonomically remote from the respective host plants of the three non-pest species of the *E. vigintioctomaculata* complex, namely thistles (Asteraceae) and blue cohosh (Berberidaceae), and (2) no local populations of the *E. vigintioctomaculata* complex have thus far been reported feeding on *S. japonense*. These points will minimize the possible interspecific bias in larval growth on a particular alternative food that would be caused by the taxonomic and/or chemical relatedness between the alternative plant and respective hosts under natural conditions. *S. megacarpum*, a closely related species to *S. japonense*, can be counted as another candidate for handy alternative foods (cf. Katakura et al., 1981) for the same reasons as those pertaining to *S. japonense*. However, the occurrence of an *E. pustulosa* population on this plant has been reported in Nopporo, Hokkaido (Katakura, 1981;

Hoshikawa, 1983; Nakano, 1985).

It should be noted that *S. japonense* does have a shortcoming when used as an alternative food. Most likely due to either photoperiodic reaction and/or thermal conditions, this plant necessarily sheds its leaves in autumn, at the latest by November. This phenological suspension of leaf supply restricts the consecutive rearing of ladybird beetles to two, or at most three, generations per year. Improvement of the culture procedure for *S. japonense* in order to ensure the leaf supply throughout year is therefore indispensable. Some authors have succeeded in consecutive rearing throughout the year in another epilachnine solanaceous-pest species, *Epilachna vigintioctopunctata* (Fabricius), with another annual solanaceous weed *Solanum nigrum* L. (cf. Kobayashi et al., 2000; Shirai and Katakura, 2000). However, when this plant, grown in Sapporo, was offered to the members of the *E. vigintioctomaculata* complex as a larval food, a rather insufficient larval performance was observed (H. Katakura, unpublished data). Although *S. nigrum* is occasionally eaten by *E. pustulosa* and *E. yasutomii* under natural conditions (Katakura, 1981; Hoshikawa, 1983), some variation with respect to its quality as a larval food for ladybirds is assumed; it may even be inappropriate larval food, especially for species belonging to the *E. vigintioctomaculata* complex.

Besides the use as a larval food, *S. japonense* has also been used repeatedly as an alternative adult food for the members of the *E. vigintioctomaculata* complex (Fujiyama and Katakura, 1997, 2001, 2002; Koizumi et al., 1999). Even though adult beetles fed preferentially on *S. japonense* (Katakura and Hosogai, 1997), and a sufficient amount of fertile eggs were produced on *S. japonense* (N. Fujiyama, unpublished data), the suitability of *S. japonense* as an adult food has not yet been precisely investigated. In addition to the larval performance examined in the present study, adult performance on *S. japonense* should be investigated in future studies.

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